CONSEQUENCES OF PROLONGED AFFERENT STIMULATION OF THE RAT FASCIA DENTATA: EPILEPTIFORM ACTIVITY IN AREA CA3 OF HIPPOCAMPUS

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Abstract—Following prolonged stimulation of the perforant path input to the dentate gyrus, long-lasting changes occur in the synaptic responses and cell properties of cells in the fascia dentata. The present study describes the effects of sustained stimulation on the major population of cells innervated by the dentate granule cells: area CA3 pyramidal cells of hippocampus. In 46% of slices from rat, sustained stimulation of perforant path was followed by spontaneous, synchronized, rhythmic bursting activity in area CA3 pyramidal cells that was evident for several hours. These bursts could be recorded extracellularly in the pyramidal cell layer, throughout the hilar region, and even in the granule cell layer.

With intracellular recording, all of the cells of the fascia dentata were found to be affected by the pyramidal cell bursts. Hyperpolarizing, inhibitory postsynaptic potential (IPSP)-like events occurred in all granule cells tested during the CA3 pyramidal cell burst. In contrast, spiny hilar "mosaic" cells discharged synchronously with the pyramidal cells, as did some of the "fast spiking" interneurons. However, most interneurons only depolarized a few millivolts during the pyramidal cell burst.

These results show that sustained stimulation of the perforant path is followed by a period of hyperexcitability in area CA3 of the hippocampus, and that hyperexcitability in area CA3 influences the activity of the cells in the fascia dentata.

In the preceding paper,43 it was shown that sustained stimulation of the perforant path input to the dentate gyrus20 led to long-lasting changes in the synaptic responses and cell properties of granule cells, as well as of other cells in the adjacent hilar region. Paired-pulse inhibition in granule cells was reduced following sustained stimulation, and multiple population spikes were evoked by single stimuli. Intracellular recording revealed that most granule cells hyperpolarized during stimulation, input resistance \(R_m\) increased, and spike frequency adaptation decreased. Hilar cells, both spiny "mosaic" cells and aspiny "fast spiking" interneurons, were extremely sensitive to stimulation; both mossy cells, and some of the interneurons, depolarized greatly and lost \(R_m\) shortly after the onset of sustained stimulation.

The present study describes changes in a major population of cells innervated by the granule cells—pyramidal cells of hippocampal area CA3—following sustained stimulation. Specifically, we examined the population of pyramidal cells that reside within the blades of the fascia dentata, area CA3c.2,27 The CA3 region of hippocampus has been studied extensively for its special properties. It receives a strong mossy fiber input.6,28,33 and so is intimately connected to the activities of the dentate granule cell population. A number of studies have focused on the key role played by CA3 cells in the generation of epileptiform activity, particularly in hippocampal slices. The CA3/CA2 population has been suggested to be the pacemaker region for epileptogenesis in hippocampus.40,53,54 Modeling studies have shown that the CA3 cells are capable of generating synchronized burst activity on their own, apparently via the local recurrent excitatory circuitry that connects CA3 pyramidal cells to each other.28 Recent studies have also shown that the CA3c subregion is particularly burst-prone when the tissue is exposed to high concentrations of potassium, and tends to drive seizure-like activity in the rest of the slice.17,30,36 Under some experimental conditions, CA3 cells have been shown to be particularly susceptible to damage. Intraventricular kainic acid injections preferentially destroy CA3 pyramidal cells,39 an effect that can be blocked by lesioning the mossy fiber input from dentate to the CA3 population.5,52 In the prolonged stimulation model developed by Olney et al.30 and Sloviter,40,49 there is significant damage to parts of the CA3 population at a time when other cell types (i.e. granule cells and CA1 cells) maintain their integrity.

These data all suggest that the CA3 population is critically involved in generation of epileptiform activity in many models of epilepsy—both in vitro and in vivo. Therefore, it was of interest to examine this population more carefully in our in vitro model of prolonged stimulation, to determine not only the

Abbreviations: AP, action potential; DAP, depolarizing afterpotential; \(E_{rev}\), reversal potential; EPSP, excitatory postsynaptic potential; IPSP, inhibitory postsynaptic potential; NMDA, \(N\)-methyl-\(D\)-aspartate, \(R_m\), input resistance; RMP, resting membrane potential.
effects of stimulation on the CA3 population itself, but the consequent action of the CA3 population on other neurons in the hippocampal slice.

**EXPERIMENTAL PROCEDURES**

Procedures for preparation of slices, intracellular recording, stimulation, and data analysis, were similar to those described in the previous paper. Other procedures are described below.

In addition to intrasomatic recordings, putative intradendritic recordings were made from dendrites in the hilus. Dendrites were impaled in the region of apical or basal CA3c pyramidal cell dendrites. Dendrites were identified by their distinct firing pattern consisting of action potentials (APs) of variable amplitudes and durations (Fig. 4). Recordings were also made from putative glial cells in the hilus. These cells exhibited the following distinctive cell properties: (1) low input resistance (R_{in}), (2) short time constant, (3) high resting membrane potential (RMP), and (4) no AP evoked by either stimulation or intracellular current injection.

For intracellular injection of Lucifer Yellow, the tips of recording electrodes were backfilled in 1.5% Lucifer Yellow CH (Sigma) in 1 M LiCl. To inject dye, 1–2 nA hyperpolarizing d.c. current was passed for a total of 10–30 min. Slices were fixed and processed as described previously.

Reversal potentials of spontaneous and evoked granule cell hyperpolarizations were determined by measuring the amplitude of the hyperpolarization at several membrane potentials, and using linear regression to calculate the reversal potential. Responses were measured from the baseline to the peak.

**RESULTS**

**Effects of sustained stimulation on pyramidal cells**

In 10 experiments, the effects of sustained intermittent stimulation were tested while recording simultaneously from area CA3c pyramidal cells intracellularly and the granule cell layer extracellularly.

Two types of responses to stimulation were recorded, and were related to the distance of the recorded pyramidal cell from the stimulation site (Fig. 1A, B). Pyramidal cells that were located relatively close to the stimulating electrode (within 1 mm; "close cells"; n = 5) were greatly affected by sustained stimulation (Fig. 1, left). These cells depolarized during the high frequency period of sustained stimulation, and remained depolarized after stimulation stopped (Table 1). Stimulus-evoked APs were of lesser amplitude than pre-stimulus APs, and were followed by large depolarizing afterpotentials (DAPs; 0–10 mV).

![Fig. 1. Effects of sustained stimulation on pyramidal cells of area CA3c. Left: responses to sustained stimulation of an area CA3c pyramidal cell that was impaled relatively close to the stimulating electrode. (A) Extracellular recording in the granule cell layer before (PRE) and after (POST) sustained intermittent stimulation. The 20 Hz response (center) was elicited in the middle of a 5-s, 20 Hz period of stimulation. Paired-pulse inhibition present pre-stimulation was absent post-stimulation. (B) Intracellularly-recorded response to a single stimulus before and after sustained stimulation. The intracellular and extracellular responses during 20 Hz stimulation were recorded simultaneously. The intracellular and extracellular responses before and after stimulation were not recorded simultaneously. The cell depolarized 10 mV during sustained stimulation and remained depolarized after sustained stimulation ended. Right: responses to sustained stimulation of an area CA3c pyramidal cell that was impaled relatively far from the stimulating electrode. Extracellular and intracellular traces represent periods as above. Two different responses to stimuli in the middle of a 5-s, 20 Hz period of stimulation are shown. During this 5-s, 20 Hz period the cell alternated between a subthreshold and a suprathreshold response to the stimulus. This cell depolarized 10 mV during stimulation but did not remain depolarized. The extracellular and intracellular recordings in C were not made simultaneously. In this and all other figures, stimulus artifacts are clipped and marked by the dots. (C) Electrode arrangement for the experiments shown above. Extracellular recording electrodes and stimulating electrodes were in similar positions but the intracellular electrode was either close to (left) or far from (right) the stimulation site. PCL, pyramidal cell layer; GCL, granule cell layer.](image-url)
Responses of area CA3 to prolonged dentate stimulation

Table 1. Cell properties of area CA3c pyramidal cells before and after sustained stimulation

<table>
<thead>
<tr>
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<th>Cells near to the stimulating electrode</th>
<th>Cells far from the stimulating electrode</th>
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<tr>
<td></td>
<td>(n = 5)</td>
<td>(n = 5)</td>
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<tr>
<td>RMP (mV)</td>
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<td></td>
</tr>
<tr>
<td>Mean</td>
<td>-65.0</td>
<td>-64.7</td>
</tr>
<tr>
<td>S.E.M.</td>
<td>2.3</td>
<td>1.5</td>
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<tr>
<td>$R_{sh}$ (MO)</td>
<td></td>
<td></td>
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<tr>
<td>Mean</td>
<td>55.1</td>
<td>50.7</td>
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<tr>
<td>S.E.M.</td>
<td>4.5</td>
<td>1.8</td>
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<tr>
<td>AP amplitude (mV)</td>
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<tr>
<td>Mean</td>
<td>81.2</td>
<td>80.1</td>
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<td>S.E.M.</td>
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Cells were assessed prior to sustained stimulation (Pre) for cell properties. Then the perforant path was stimulated until there was a long-lasting reduction in paired-pulse inhibition of the granule cell population spike. At that point, cells were reassessed for cell properties post-stimulation (Post).

Fig. 1B). The $R_{sh}$ of these cells was also reduced following sustained stimulation (Table 1). None of these effects had reversed when the cells were examined intracellularly, all pyramidal cells (n = 20) burst during the extracellular discharge; when pyra-
Intracellular recordings from pyramidal cells showed that for a given cell, each burst was similar to the next in the number of APs, and amplitude and duration of depolarization. However, there was some variability in the form of the burst from cell to cell within a slice, as well as from slice to slice.

In three slices where the time course of development of bursting activity was monitored, bursts were first detected 0.5–2 h following sustained stimulation. The first bursts were relatively short (< 100 ms) and burst frequency was slow (up to 0.04 Hz). Within 2 h following detection of spontaneous bursts, bursts had become larger and burst frequency reached a peak. By 6 h after sustained stimulation burst frequency decreased and eventually bursts could no longer be detected. It is important to note that the decrease in burst frequency could be due to general deterioration of slices, which normally begins 6–8 h after dissection.

Extracellular recording revealed that large field potentials occurred throughout the entire CA3 region of the slice (i.e. in area CA3a, b and c), and throughout the hilar region during spontaneous bursts of pyramidal cells, and could even be recorded in the granule cell layer (Fig. 3). (Most of these slices were cut longitudinally, so the spread of bursting activity to area CA1 could not be determined.) A depth profile, extending from the CA3c pyramidal cell layer to the granule cell layer, showed that the positivity recorded in the pyramidal cell layer, upon which population spikes were superimposed, changed polarity in the pyramidal cell dendritic region (Fig. 3). A mixed negative and positive potential was recorded in the proximal dendrites and a pure negativity occurred in distal dendritic regions and in the granule cell layer. A similar pattern of field potentials was recorded when depth profiles were made in the pyramidal cell basal dendrites and lower blade of the dentate gyrus. No field potential was recorded in the granule cell dendritic layer (stratum moleculare) during spontaneous bursts in area CA3c. In all cases, single stimuli to the perforant path evoked field potentials that were similar to spontaneous events.

Since field potential recordings suggested the presence of a current sink in the area of pyramidal cell dendrites (Fig. 3), we used intradendritic recording to examine the possibility that spontaneous bursting originated in pyramidal cell dendrites. Many spontaneous depolarizations recorded intradendritically coincided with bursts recorded simultaneously from pyramidal cell somata (Fig. 4). In addition, there were many smaller spontaneous depolarizations that did not coincide with activity recorded intrasomatically. However, it was not possible to determine definitively whether the somatic events were triggered by the dendritic events, or vice-versa. The spontaneous dendritic depolarizations were quite variable in amplitude and duration, and did not vary systematically with distance from the pyramidal cell layer.

When Lucifer Yellow was injected while recording from putative pyramidal cell dendrites, pyramidal cells were stained (Fig. 4, n = 2), verifying that these recordings were from pyramidal cell dendrites and not from hilar cells.

Since pyramidal cell bursts could be detected as far away from the pyramidal cells as the granule cell layer, we investigated whether some of the late population spikes recorded in the granule cell layer following sustained stimulation might reflect AP discharge of pyramidal cells. The nature of these late population spikes had been puzzling, because simultaneous recording of granule cells and the granule cell population spike showed that granule cells did not fire APs that coincided with the late population spikes. In five slices where this question was investigated, it was found that pyramidal cell APs coincided with late population spikes recorded in the granule cell layer (n = 5 pyramidal cells; Fig. 5). In contrast, when intracellular recordings were made from granule cells in these slices (n = 8), granule cell APs did not coincide with the late population spikes (Fig. 5). The granule cells that were studied were impaled close (within 100 μm) to the extracellular recording electrode, as well as other areas in the granule cell layer.

Effects of sustained stimulation on cells of the fascia dentata

While recording from area CA3 pyramidal cells extracellularly or intracellularly, simultaneous intracellular recordings were made from different cells of the fascia dentata: granule cells, spiny hilar "mossy" cells, "fast spiking" aspiny interneurons, and
putative glial cells. All of these recordings were made after sustained stimulation had blocked granule cell paired-pulse inhibition, and in slices where area CA3c pyramidal cells were bursting spontaneously.

Granule cells. While CA3 bursting activity was monitored in the pyramidal cell layer, 34 granule cells were impaled. During spontaneous pyramidal cell bursts there was little evidence of any spontaneous granule cell activity at RMP, but when granule cells were depolarized with d.c. current, a hyperpolarization occurred during the burst (Fig. 6).

These hyperpolarizations were typically monophasic events, and the onset of the hyperpolarizations coincided with the onset of the population bursts or intracellularly recorded pyramidal cell bursts (Figs 6 and 7). In 16 cells, the granule cell hyperpolarization outlasted the extracellular burst recorded in CA3c. The hyperpolarizations were difficult to reverse; in most cases there was no detectable reversal, even at very hyperpolarized potentials (i.e. −85 to −90; it was difficult to pass enough current to hyperpolarize cells beyond −90 mV). The cell shown in Fig. 7B was an exception, in that a measurable response reversal occurred at a membrane potential close to RMP (RMP = −76 mV). In four other cells where direct demonstration of reversal potential (E_{rev}) of the hyperpolarization was possible, mean E_{rev} (± S.E.M.) = −72.5 ± 1.3 mV. In five cells, there was clear evidence of a two-component hyperpolarization with early and late phases. A clear reversal of the late phase of the response was not possible in these cells;
the mean extrapolated reversal potential in these cells was $-96.1 \pm 4.3$ mV.

**Mossy cells.** Hilar cells with the electrophysiological properties of mossy cells were difficult to detect in slices where pyramidal cells burst spontaneously. When mossy cells were impaled ($n = 15$) they had a low RMP (more positive than $-50$ mV), low $R_m$ (< 10 MΩ), and short APs (< 40 mV). These observations are consistent with our previous report that mossy cells are very sensitive to sustained stimulation, and appeared to be damaged (or impalements lost completely) by the sustained intermittent stimulus paradigm used here. During pyramidal cell bursts, mossy cells also burst; the bursts were synchronous with pyramidal cell discharge, but mossy cell bursts typically outlasted the pyramidal cell burst (Fig. 8).

**Interneurons.** Detection of hilar cells with the electrophysiological characteristics of interneurons was also difficult after sustained stimulation, in slices where pyramidal cells burst spontaneously; this observation is also consistent with previous studies. Only two hilar interneurons were impaled that had an RMP more negative than $-50$ mV. In contrast, 12 interneurons with RMPs more negative than $-50$ mV were impaled on the border of the granule cell layer and the hilus.

The interneurons displayed two types of response during the bursts of area CA3c pyramidal cells, and these responses correlated with the sensitivity of the interneurons to stimulation of the perforant path. Ten out of 12 interneurons tested with single stimuli to the perforant path were relatively “insensitive” in that they had a stimulus threshold similar to that of granule cells. These relatively “insensitive” cells depolarized slightly (mean $\pm$ S.E.M. = $4.1 \pm 1.2$ mV; $n = 5$), or fired one to four APs ($n = 7$) during pyramidal cell bursts (Fig. 9). No hyperpolarizations were found, even if these interneurons were depolarized with d.c. current. All of these cells were located on the border of the granule cell layer and the hilus.

The other type of interneuron response seen during the pyramidal cell burst was recorded from relatively “sensitive” cells ($n = 2$). These cells were considered sensitive because their discharge threshold was far below the threshold for a granule cell population spike (Fig. 10). In these sensitive cells, a burst occurred synchronously with the pyramidal cell burst (Fig. 10). One of the sensitive cells was located in the hilus and the other was located on the border of the granule cell layer and the hilus.

**Glia.** Impalements of putative glia were found regularly in the CA3 pyramid cell layer and hilar region prior to and following sustained stimulation. In all glia that were impaled prior to sustained stimulation, no spontaneous changes of membrane potential occurred ($n = 10$). However, in eight glial cells examined following sustained stimulation, long-lasting (over 1 s) depolarizations occurred during pyramidal cell bursts. When glia were impaled in the pyramidal cell layer, these depolarizations were large (up to 20 mV; Fig. 11A); when recording from glia in the hilus the depolarizations were also long-lasting, but were much smaller in amplitude (maximal amplitude = 5 mV; Fig. 11B). Glial depolarizations always

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Fig. 5. (A and B) Simultaneous recordings from the granule cell layer (A; extracellular) and from a granule cell (B; intracellular), prior to (PRE STIM) and following (POST STIM) sustained stimulation. Following sustained stimulation there were multiple population spikes recorded in the granule cell layer that did not correspond to the APs of the granule cell. Vertical calibration: 5 mV for extracellular records (A); 15 mV for intracellular records (B and C). (C) A pyramidal cell was impaled in the same slice immediately after the recordings in A and B were made. The same stimulus intensity and paired pulse interval used in A and B elicited APs in the pyramidal cell that coincided with late population spikes seen in the granule cell layer extracellular record.
Responses of area CA3 to prolonged dentate stimulation

Fig. 6. Granule cells hyperpolarize during pyramidal cell bursts. (A) Simultaneous recording from a pyramidal cell (PC) and a granule cell (GC) following sustained stimulation. A depolarizing current pulse evoked APs in the pyramidal cell (arrow) with no simultaneous activity in the granule cell. During a spontaneous burst of the pyramidal cell (asterisk) there was a large hyperpolarization in the granule cell. The granule cell was depolarized with d.c. current (membrane potential = −54 mV). Capacitative artifacts of pyramidal cell APs are evident in the granule cell recording (arrowhead). (B) While recording from the same granule cell as in A, the spontaneous burst was recorded simultaneously in the pyramidal cell layer (PCL) extracellularly. Note that the onset of the granule cell hyperpolarization coincided with the onset of the extracellular burst.

Discussion

We have identified effects of sustained stimulation of the perforant path on cells of the fascia dentata as well as on pyramidal cells of hippocampal area CA3c. The differences in responses of various cell types to sustained stimulation is striking. Granule cells, hilar "mossy" cells, interneurons, and CA3c pyramidal cells all showed unique responses to the pattern of sustained stimulation used in our study.

Effects of sustained stimulation on area CA3c pyramidal cells

This study showed that CA3c pyramidal cells vary in their response to sustained stimulation of the perforant path, and this variability appeared to depend on the proximity of the impaled cell to the source of stimulation; i.e., cells that were located near the site of stimulation were affected more than cells that were located further from the stimulation site. It is not clear whether the affected CA3c cells are intrinsically more sensitive to stimulation than CA3 cells further from the hilus, or they simply receive a higher density of excitatory input. That the latter is more likely is indicated by the dependence of this effect on AP discharge during sustained stimulation. Pyramidal cells located near the stimulating electrode fired more often during sustained stimulation than pyramidal cells far from the stimulating electrode—and were more damaged after sustained stimulation. AP discharge during sustained stimulation may thus reflect the critical mechanism underlying the development of adverse effects of sustained stimulation (such as depolarization and loss of Rm). It is possible that the vulnerability of CA3c cells located near to the stimulating electrode reflects the limitations of stimulating cells in a slice preparation, where connections between cells that are far apart (i.e., granule cells and area CA3b pyramidal cells) are more likely to be severed than connections between cells that are close together (i.e., granule cells and area CA3c pyramidal cells).

The responses to sustained stimulation of area CA3c pyramidal cells that were located near to the stimulating electrode were similar to the responses of mossy cells and "sensitive" interneurons to sustained

Fig. 7. Characteristics of granule cell hyperpolarizations. (A) Simultaneous recordings from the pyramidal cell layer (extracellular, top) and a granule cell (intracellular, bottom). During the spontaneous burst recorded in the pyramidal cell layer, the granule cell hyperpolarized. This granule cell was depolarized to the level of spontaneous firing; an arrow points to a spontaneous AP that was clipped. (B) The membrane potential of the cell in A was manipulated with d.c. to reverse the spontaneous granule cell hyperpolarization. Membrane potential is shown on the left. (C) Amplitude of the granule cell hyperpolarization is plotted against membrane potential for the cell shown in A and B. The reversal potential for the hyperpolarization was −70.8 mV.
stimulation. During the high frequency periods of sustained stimulation, these CA3c cells depolarized, AP amplitude decreased, and APs broadened. Following sustained stimulation these CA3c cells were depolarized, \( R_m \) decreased and AP amplitude decreased. However, despite qualitative similarities, the degree of change in the area CA3c pyramidal cells located near the stimulating electrode was very different from that seen in mossy cells and “sensitive” interneurons. For example, during high frequency stimulation, the fast AP of pyramidal cells decreased in amplitude, whereas the fast AP was lost completely in most mossy cells and “sensitive” interneurons. Further, following sustained stimulation, CA3c cells were less depolarized and suffered a smaller decrease in \( R_m \) than mossy cells and “sensitive” interneurons.

These results from our in vivo preparation are consistent with the data obtained from sustained stimulation in vivo, 34,44,45 where some pyramidal cells in the CA3c region were sensitive to stimulation and others were not. Our data are also consistent with the hypothesis that the intracellular supply of calcium-binding proteins is related to the susceptibility of neurons to sustained stimulation. 42,50,51 CA3 neurons lack calcium-binding proteins 4,50,51 and were sensitive to sustained stimulation. However, other factors besides calcium-binding proteins must be involved, since some CA3 cells were relatively insensitive to sustained stimulation, both in vivo and in vitro.

Synchronized bursts of area CA3c following sustained stimulation

Following sustained stimulation, dramatic changes were found in the activity of area CA3c—spontaneous, rhythmic, synchronized bursts occurred. The mechanisms underlying initiation of the bursts are unclear, but several factors may contribute. One important factor may be a decrease in inhibition, as reflected in the loss of paired-pulse inhibition in the granule cell layer. Such a loss was observed in the granule cell layer of all slices prior to development of bursting in the pyramidal cell layer. In addition, in some slices, a loss of paired-pulse inhibition was evident while recording from interneurons, mossy cells, or pyramidal cells during sustained intermittent stimulation. Certainly, many studies suggest a role of decreased GABAergic inhibition in epileptogenesis. 35,34,36

The fact that all bursts could be elicited by stimulation of the perforant path, and that large excitatory postsynaptic potentials (EPSPs) were uncovered in hyperpolarized, bursting pyramidal cells, suggest that excitatory synaptic factors may also be involved. In particular, synapses in the hilar region—presumably
Fig. 10. Interneurons that are relatively sensitive to stimulation of the perforant path burst during the pyramidal cell burst. (A) Simultaneous extracellular recording in the pyramidal cell layer (top) and intracellular recording from an electrophysiologically identified interneuron (bottom). The interneuron burst coincided with the spontaneous burst in the pyramidal cell population. Inset: the response of the interneuron in A to a 0.5-nA, 100-ms depolarizing current pulse. (B) Responses to a pair of identical stimuli to the perforant path are shown for an extracellular recording in the granule cell layer (top) and (simultaneously) in the same interneuron as in A (bottom). The interneuron shown in A was a relatively sensitive interneuron, since a stimulus that was subthreshold for the granule cell population spike was far over threshold for interneuron discharge. The interneuron was located 300 μm further from the stimulating electrode than the extracellular recording site in the granule cell layer. Vertical calibration: 5 mV for A and B, top traces; 15 mV for A and B, bottom traces; 30 mV for inset in A. APs are truncated by spike digitization.

from granule cells onto pyramidal cell dendrites—may be important. The extracellularly-recorded burst potential showed a peak negativity in the hilar region, suggesting that synapses onto dendrites in this region were driving the burst. Although the granule cells did not fire repetitively themselves, the release of transmitter from mossy fiber terminals produces extremely large EPSPs on pyramidal and mossy cells (unpublished observations), which could be sufficient to trigger recurrent excitatory circuits in a slightly disinhibited CA3c region.28,34,53,58

Other factors contributing to burst generation (see also Ref. 53) include a disruption of regulation of the ionic microenvironment. The observation that glial cells depolarize during pyramidal cell bursts suggests that there was a large rise in extracellular potassium during this discharge. Although such a change in extracellular K⁺ concentration may simply reflect repetitive discharge (i.e. a result rather than a cause of the burst), investigators have suggested that abnormal increases in the concentration of extracellular potassium may contribute to epileptogenesis.11,17,38,39 Even a small chronic elevation in extracellular potassium in the CA3c region, due to the sustained stimulation, could generate CA3 bursting. Traynelis and Dingledine60 demonstrated that synchronized CA3c bursts could be initiated by raising potassium concentration in the medium bathing their slices.

Excessive calcium entry into CA3 neurons may also be involved in burst production. It has been shown that there is a large drop in the extracellular concentration of calcium during hippocampal stimulation,5,13,21 and such changes in extracellular calcium levels have been hypothesized to be critical to the development of epileptiform activity.61 Calcium may enter cells through voltage-dependent calcium channels, or through receptor-activated channels, controlled by the N-methyl-d-aspartate (NMDA) receptor. An involvement of NMDA receptors in burst generation seems likely, since the effects of sustained stimulation were seen only when we perfused the tissue with a medium containing a low magnesium concentration.62 NMDA receptor modulation of the channel is blocked by magnesium ions.23

There are, however, several questions concerning the mechanisms underlying spontaneous bursting activity in area CA3 that calls into question the importance of direct, stimulus-induced changes in the microenvironment. In particular, bursts developed with a considerable delay after sustained stimulation, a period during which ionic concentrations could easily have been restored to normal levels. It is unclear what occurs during the period between the end of stimulation and the onset of bursting that may be necessary for the development of hyperexcitability. In this regard, sustained stimulation is similar to some experimental models of ischemia, where there is a significant delay after the ischemic episode before all of the effects are manifest.16 Experimental ischemia is also similar to sustained stimulation in that hilar cells appear to be particularly sensitive to the ischemic insult relative to other hippocampal cells.8 These similarities suggest that ischemic damage and damage due to sustained stimulation have similar underlying mechanisms. In both experimental
situations, it remains unclear why hilar neurons are so susceptible to injury, and whether hilar cell degeneration is critical to the development of long-lasting changes in the pathophysiology of the hippocampus.

Activity of cells in the fascia dentata during spontaneous pyramidal cell bursts

We found that every cell type that was impaled in the fascia dentata was affected by the pyramidal cell burst. This was unexpected, since the majority of projections of pyramidal cells are to area CA1 and contralateral area CA3. However, pyramidal cells do have extensive axon collaterals, and these collaterals may activate local neuronal circuits, resulting in the various types of activity observed in the fascia dentata during pyramidal cell bursts. In addition, increases in the concentration of extracellular potassium during synchronized pyramidal cell bursts may depolarize nerve terminals and help activate cells in the region, even if they do not receive excitatory input directly from pyramidal cells. Our data from putative glia of the hilus and pyramidal cell layer are consistent with this hypothesis; the substantial depolarizations of glial cells presumably reflect increases in extracellular potassium concentration throughout area CA3 and the hilus during pyramidal cell bursts.

Granule cells and interneurons. In the case of granule cells, mono- or biphasic hyperpolarizations occurred during pyramidal cell bursts. When reversal potentials were calculated, they closely estimated the equilibrium potentials for chloride or for potassium, suggesting that these ions are involved in the hyperpolarizations. In hippocampus and other areas, the GABA receptor is associated with a chloride channel, and GABAA receptors with a potassium channel. GABAAergic interneurons of fascia dentata make numerous contacts on granule cells, and GABA is likely to be the neurotransmitter that mediates the hyperpolarizations of granule cells during pyramidal cell bursts. Activation of GABAAergic interneurons may be due to their excitation by pyramidal cells; these interneurons then make inhibitory synapses on granule cells. Although there is no direct anatomical evidence of pyramidal cell excitatory synapses on interneurons of the fascia dentata, there are precedents for this type of local circuitry, since hippocampal pyramidal cells are known to make excitatory synapses on local inhibitory interneurons. Our data are consistent with the hypothesis that excitation of inhibitory interneurons by pyramidal cells underlies the granule cell hyperpolarization, in that some interneuron-like cells were greatly excited during pyramidal cell bursts. However, most of the interneuron-like cells that we monitored depolarized only slowly and weakly. Since the onset of the granule cell hyperpolarization was extremely fast relative to CA3 bursts, it is unlikely that the activity of these less responsive interneurons was responsible for the hyperpolarization of granule cells. Unfortunately, we found few of the other interneuron type that was greatly excited at short latency; indeed, these “sensitive” interneurons appeared to suffer injury during stimulation. Thus, the source of inhibitory input to the granule cells remains somewhat of a mystery.

Our data suggest a heterogeneity of function among interneurons. Anatomical studies suggest that there are different groups of interneurons in the fascia dentata, and our data tend to separate them into two functional groups: (1) interneurons that are located throughout the fascia dentata, that are relatively “sensitive” to stimulation of the perforant path, that depolarize greatly during pyramidal cell spontaneous bursts, and serve to hyperpolarize granule cells, and (2) interneurons that are located on the border of the granule cell layer, that are relatively “insensitive” to perforant path stimulation, that depolarize weakly during pyramidal cell spontaneous bursts, and that do not provide a powerful hyperpolarizing drive to the granule cells during the pyramidal cell spontaneous bursts. Alternatively, the differences we recorded between interneurons may be due to the extent that excitatory inputs to interneurons were severed during slice preparation.

Mossy cells. Since the cell properties and synaptic responses of mossy cells deteriorated during prolonged stimulation, it was not surprising that mossy cell impalements were rare following sustained stimulation; cell properties of the impaled cells were inferior to those of mossy cells from unstimulated slices. These results suggest that although sustained stimulation may have deleterious effects on mossy cells, it does not lead to a complete loss of cell integrity—at least for some proportion of the mossy cell population. Some mossy cells may be more resistant to sustained stimulation than others, due to, for example, differences in Na+/K+ pump activity. Further, differences in the amount of deafferentation of mossy cells during slice preparation might isolate certain mossy cells more than others from their inputs, and these deafferented mossy cells might be protected from the effects of sustained stimulation.

Mossy cell bursts were consistently longer-lasting than the pyramidal cell bursts recorded in the same slice. This observation may be explained by a greater mossy cell sensitivity to the burst trigger, or a larger and longer duration input to this cell population. Perhaps as likely, the long duration mossy cell bursts may be attributed to the fact that these cells were depolarized (following sustained stimulation), and less able to repolarize after the burst had begun than the relatively healthy pyramidal cells. One of the effects of sustained stimulation on mossy cells might be to impair regulatory mechanisms (such as the activation of K+ conductances and ion pumps) that would normally protect cells from the effects of strong depolarizing stimuli.
The suggested circuitry underlying granule cell hyperpolarizations discussed above implied that pyramidal cells drive the bursting activity. However, it is also possible that mossy cells constitute the cell population that drives the bursts, since the mossy cells and pyramidal cells appeared to burst simultaneously. The near simultaneity of the bursts suggests a close synaptic relationship between the two cell populations. However, there have been no reports of direct connections of either CA3 pyramidal cells to mossy cells or mossy cells to CA3 pyramidal cells, and dual recordings from mossy cells and pyramidal cells in the present study did not show whether one cell type was driving the other. It is certainly possible that the two cell populations merely share similar excitatory inputs. If that is the case, however, neither pyramidal cells nor mossy cells are the “drivers” of bursting activity; the generator would still need to be identified.

CONCLUSION

We have shown that cells of the fascia dentata and hippocampus are profoundly affected by sustained stimulation of the perforant path, supporting similar studies in vitro. Furthermore, we have established through intracellular recording the different responses of various cell types to sustained stimulation. These studies offer a unique experimental model in which to study the development of hyperexcitability, and may also be useful in understanding the basis of selective neuronal vulnerability. Finally, these studies may contribute to our understanding of the cellular organization and interaction of the hippocampus and the fascia dentata.

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